Exudate flavonoids from *Artemisia* (Asteraceae-Anthemideae): structural characterization by HPLC-UV and recorded UV-spectra

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Introduction

Exudate flavonoids are produced by glandular trichomes in mostly higher evolved families such as the Asteraceae and Lamiaceae (for information on general aspects on exudate flavonoid formation see Valant-Vetschera & Brem, 2006). Exudates obtained from *Artemisia* species consist of different derivatives, which are based upon flavones, flavonols, flavanones and dihydroflavonols, in addition to various terpenoids (Valant-Vetschera et al., 2003; Bhutia & Valant-Vetschera, in press). In the framework of chemodiversity research, studies on several accessions within a single species, and comparison with other species, call for a method allowing comparative analysis on a larger scale. Since flavonoid compounds are UV-sensitive, HPLC coupled with UV diode array detector is the method of choice. Comparative analysis of new exudates is based upon the retention times, coupled with the recorded UV-spectra. In addition, standard reaction steps (shift reactions) according to Mabry et al. (1970), which have to be done separately with isolated compounds, provide valuable structural information. The value of retention times, UV-spectra and TLC-properties of flavonoids in terms of analytical information was addressed recently by Greenham et al. (2003). Only very few of the compounds analyzed now are contained in this paper, which gives numerical values only.

The focus of this presentation therefore lies in characterizing 31 flavonoids obtained as more or less pure reference compounds from the lab of Prof. Wollenweber (TU

Darmstadt, Germany), both with respect to their HPLC properties and their characteristic UV absorption spectra, also in graphical form. Fifteen of these compounds were pure enough to be analyzed separately by adding the diagnostic reagents which indicate the position of possible substituents on the core molecule. These analyses were carried out on a UV-spectrophotometer (Specord 205, Analytik Jena). The shift reactions were performed according to Mabry et al. (1971). Since the main purpose was to provide illustrations of the UV spectra, to be used for comparison with HPLC-generated spectra, discussion as to the behaviour of flavonoids with specific substitution patterns was omitted. This kind of information may be obtained from Mabry et al. (1970) and Greenham et al. (2003).

Methods and Results

The flavonoids studied here belong to the following subclasses (for formulae see Fig. 1):

a) Flavones
b) Flavonols
c) Flavanones

Derivatives mostly contain at least one methoxy group, more often several replacing the OH-groups and/or introducing OH at position 6 of the core molecule. Dihydroflavonols could not be studied at present due to lack of material. The exudate profiles known so far from the genus have been discussed previously. (Valant-Vetschera et al., 2003). This study revealed that corresponding subclasses of flavonoids also exhibited similar substitution patterns, indicative of common biosynthetic activities.

Information to the general procedure of HPLV-separation is available from Table 1 (retention times of single flavonoids), while Table 2a and 2b inform about the technical parameters (solvent systems). Since not all of the compounds could be separated by a single system, two systems had to be used for efficient separation and identification. Figure 2 illustrates the HPLC chromatogram combined with UV-spectra of compounds stored in a database, to provide an idea about the application of the system now described. All of the spectra have been converted to pdf-files. Appendix 1 contains HPLC borne UV-spectra, while Appendix 2 contains the separately analyzed UV spectra including the characteristic shift reactions caused by

standard reagents. The reaction spectra were recorded separately and then combined electronically by using Adobe Illustrator. Retention times are only comparable if the same parameters apply in HPLC analysis as specified in this publication.

References

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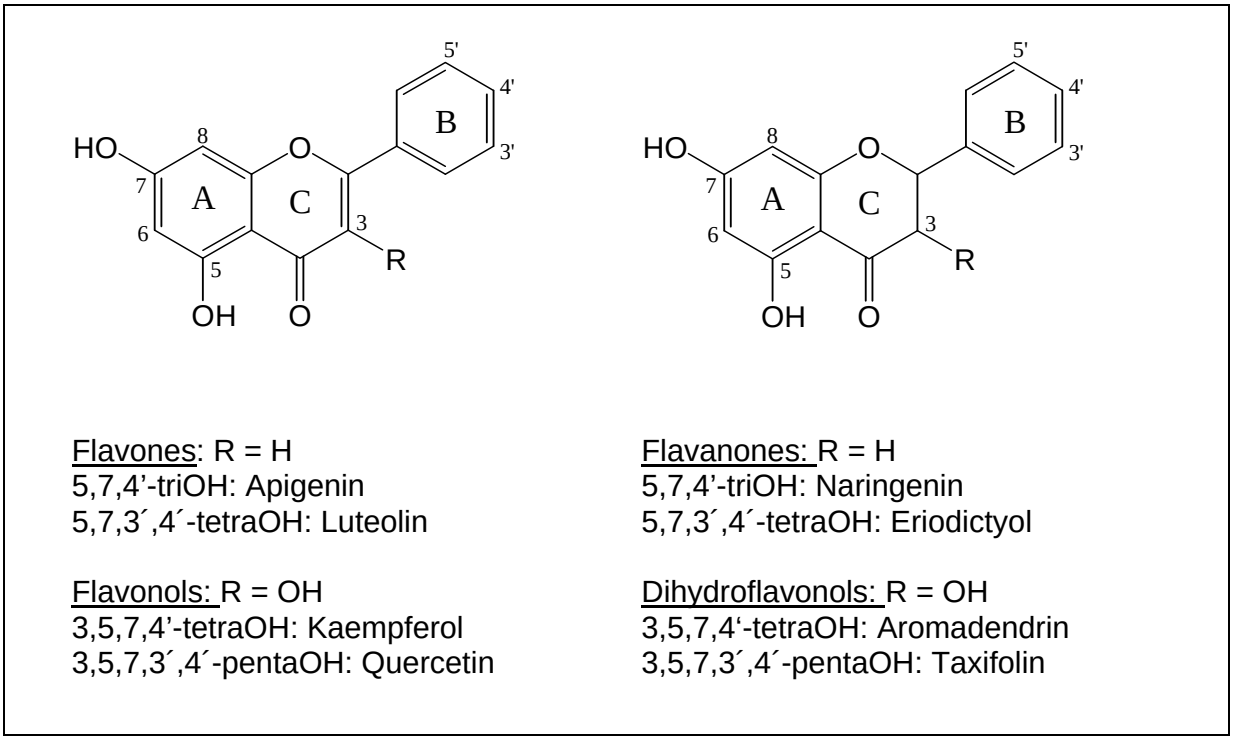

Flavones: R = H
5,7,4'-triOH: Apigenin
5,7,3´,4´-tetraOH: Luteolin

Flavonols: R = OH
3,5,7,4'-tetraOH: Kaempferol
3,5,7,3´,4´-pentaOH: Quercetin

Flavanones: R = H
5,7,4'-triOH: Naringenin
5,7,3´,4´-tetraOH: Eriodictyol

Dihydroflavonols: R = OH
3,5,7,4'-tetraOH: Aromadendrin
3,5,7,3´,4´-pentaOH: Taxifolin

Fig. 1 Basic flavonoid structures of *Artemisia*.

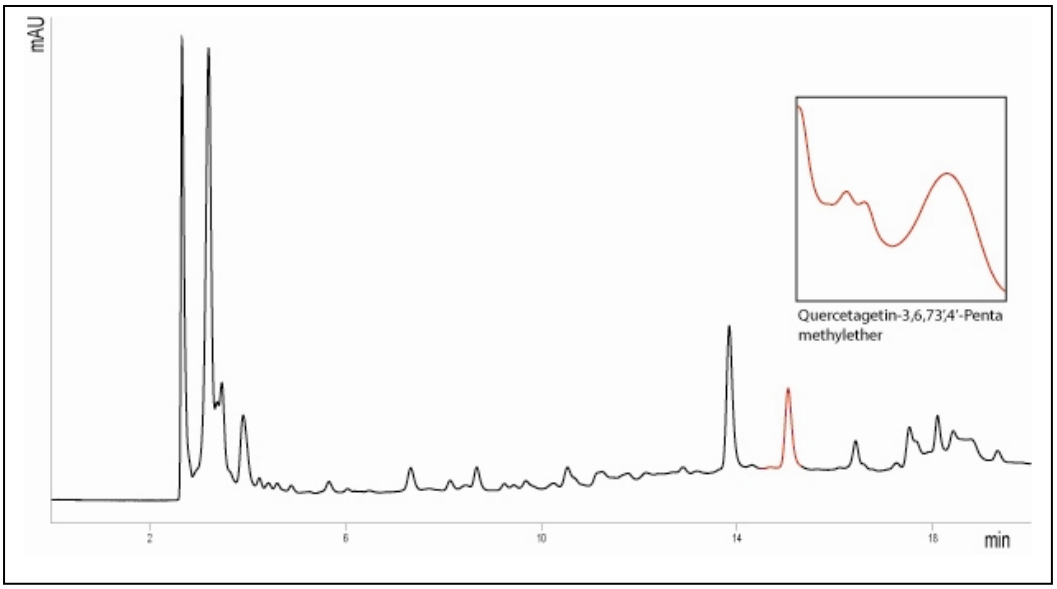

Abb. 2 *Artemisia absinthium*, HPLC-Profile and UV-Spectra from HPLC

Flavonoids		Retention time (Min.)	
No.	**Flavones**	**Method A**	**Method B**
1	Apigenin-7-Methyl ether	17.107	
2	Scutellarein-6-Methyl ether	13.316	
3	Scutellarein-6,7-Dimethyl ether	15.353	
4	Scutellarein-6,4'-Dimethyl ether	17.413	
5	Scutellarein-6,7,4'-Trimethylether	19.690	
6	Luteolin-4'-Methyl ether	13.390	
7	Luteolin-7,3'-Dimethyl ether	13.628	
8	Luteolin-7,4'-Dimethyl ether	17.428	
9	6-OH-Luteolin-6,3'-Dimethyl ether	17.405	
10	6-OH-Luteolin-6,7,3'-Trimethyl ether	15.637	
11	6-OH-Luteolin-6,7,4'-Trimethyl ether	15.626	
12	6-OH-Luteolin-6,7,3',4'-Tetramethyl ether	17.864	
	Flavonols		
13	Kaempferol-3,4'-Dimethyl ether	20.183	
14	6-OH-Kaempferol-3,6,7-Trimethyl ether	18.387	
15	6-OH-Kaempferol-3,6,4'-Trimethyl ether	16.180	
16	Quercetin-7-Methyl ether	15.252	
17	Quercetin-3,3'-Dimethyl ether	14.149	
18	Quercetin-3,7,3'-Trimethyl ether	20.569	
19	Quercetagetin-6-Methyl ether	11.752	
20	Quercetagetin-3,6-Dimethyl ether	12.271	
21	Quercetagetin-3,6,7,3'-Tetramethyl ether	16.406	
22	Quercetagetin-3,6,7,4'-Tetramethyl ether	16.442	
23	Quercetagetin-3,6,7,3',4'-Pentamethyl ether	18.748	
	Flavanones		
24	Naringenin		5.693
25	Naringenin-7-Methyl ether		9.440
26	Naringenin-4'-Methyl ether		9.623
27	Naringenin-7,4'-Dimethyl ether		14.290
28	Eriodictyol	12.144	
29	Eriodictyol-7-Methyl ether		7.446
30	Eriodictyol-3'-Methyl ether		5.613
31	Eriodictyol-7,3'-Dimethyl ether		9.973

Table1: Retention times of flavonoids analyzed

Time (Min.)	Acetonitril (Vol. %)	Reisch-Buffer (Vol. %)
0,01	30	70
17	90	10
20	100	-
28	100	-

Table 2a: Solvent Gradient Method A

Time (Min.)	Methanol (Vol. %)	Reisch-Buffer (Vol. %)
0,01	55	45
17	90	10
20	100	-
28	100	-

Table 2b: Solvent Gradient Method B

MeOH	**Methanol**
$AlCl_3$	**Aluminium chloride**
HCl	**Hydrochloric acid**
NaOAc	**Sodium acetate**
H_3BO_3	**Boric acid**
NaOMe	**Sodium methoxide**
sh	**shoulder**

Abbreviations to reaction spectra